Project Report No. 440

Abstract and Summary

October 2008

Validation of a model to avoid conditions favouring Ochratoxin A production during ambient-air drying

by

T J Wontner-Smith[1], D M Bruce[2], D M Armitage[1], S K Cardwell[1] and P Jennings[1]

[1]Central Science Laboratory, Sand Hutton, York YO44

[2]David Bruce Consulting Ltd, 54 High Road, Shillington, Hitchen, Herts SG5 3LL

This is the final report of an HGCA project which started in July 2005 for 38 months and was funded by a contract from HGCA of £142,976 (Project No. 3133).

Contents

ABSTRACT

Ochratoxin A (OTA) is a fungal mycotoxin that may form when grain moisture content exceeds 18%. EU food limits are set at 5ppb. In a previous HGCA project the risk of OTA formation during drying was assessed using the simulation tool 'Storedry', substituting the old biodeterioration criteria of visible mould or germination loss with a new model for avoiding OTA formation. This model was based on the time taken before fungal growth enters the rapid growth phase. Shorter safe storage times were predicted using the new model. The present study aimed to compare these predictions with real, full scale drying processes.

Four bins each of 30 tons of wheat were dried using ambient air in two seasons. Physical parameters (e.g. moisture and temperature in the wheat and biological changes including mycotoxin production) were measured to provide data to validate the model. Valuable data on changes in populations of fungi were also collected.

The simulation, 'Storedry', gave good predictions of the general drying behaviour and final moisture content. However, it underestimated drying time by about 20%, mainly because the exhaust air in the simulation was less saturated than in the experiment and so the prediction of exhaust humidity by Storedry needs to be improved.

The prediction of biodeterioration using the new OTA model was found to be too rapid. Experimental data indicated a safe storage time at least two times that predicted by the model even when the wheat had been inoculated with *Penicillium verrucosum*, the main cause of OTA in UK cereals.

Based on this, simulations were run with safe storage times increased by a factor of two. From these simulations it was concluded that, for drying with continuous ventilation, the new OTA model was no more demanding than the well established model based on visible mould and significant loss of viability. Therefore, when drying by continuous ventilation, the risk of OTA appears no greater than the risk of visible mould or significant loss of viability.

For drying by continuous ventilation, the time for the drying front to pass through the bed does not need to be reduced. Hence, recommendations for bed depth and airflow rate in The Grain Storage Guide (HGCA, 2003) remains appropriate for continuous ventilation.

Drying rate does not need to depend on the amount of inoculum present.

SUMMARY

Introduction

Mycotoxins are toxic chemicals produced by fungi. In grain harvested and stored in the UK the main threat of mycotoxin contamination is from ochratoxin A (OTA) which is most likely to be produced by *Penicillium verrucosum*. While *P. verrucosum* grows at 80% relative humidity (r.h.), it does not produce OTA below 85% r.h. At 25^{o}C, grain at 18.7% moisture content gives an r.h. of 85% and so grain with moisture contents below 18.7% will be safe from OTA production.

In a previous HGCA-funded project (Bruce *et al.*, 2006), the risk of OTA formation during drying was assessed using the simulation tool 'Storedry', substituting the old biodeterioration criteria of visible mould or germination loss with a new model for time before onset of rapid fungal growth and possible OTA formation. This new model was developed by Jonsson (Olsen *et al*. 2004) and was based on small scale measurements. Shorter safe storage times were predicted using this new model and near ambient drying was identified as the operation during which grain was most at risk from OTA.

The original aim of the present study was to validate the new OTA model at full scale by comparing measured toxin production with predictions by the new model. This aimed to ensure confidence in the strategies derived in the previous project using the combined model of drying and biodeterioration and indicate where improvements to the model might be appropriate.

Changes in physical parameters including temperature and moisture content, and biological changes including mycotoxin production, were observed in four beds of drying wheat in each of two drying seasons to provide data to validate the model at full scale. Valuable data on other biological changes such as populations of fungi were also collected.

Year 1

Approximately 30 tonnes of freshly harvested wheat was placed in each of four open top bins each 3x3m in plan view. Each bin was fitted with a fan suitable for ambient air drying, connected to two air ducts located just below the parallel-sided part of the bin. Two of the bins contained grain with average initial moisture contents of 19.9% and 21.4% (all moisture contents are given on a wet basis). The other two bins contained grain at higher moisture levels, with average initial moisture contents of

23.3% and 23.9%. These moisture contents had been chosen to cover the range in which OTA formation would be expected. Bed depths were 3.5 to 4m.

As the grain was dried, the temperature was monitored in each bin using thermocouples and r.h. of the air in each aeration duct and at the surface of the grain was measured. Pressure switches were placed in the duct to monitor the timing of the fan operation and the static pressure in the air ducts was measured. Temperature, pressure and r.h. data were logged every 10 minutes onto three dataloggers.

Sampling of grain at five depths, down to 2m, was done on a daily basis except at weekends and bank holidays and the sampled grain was analysed for moisture content by oven drying. Airflow was measured daily for each bin at the inlet to the fan using a vane anemometer and at five locations on the grain bed surface using a Casella meter.

Drying was continued until the moisture content of the grain close to the surface of the bin had fallen, indicating that the drying front had passed through the grain.

Samples were collected at start and end of drying for analysis of fungi and OTA.

Measurements of moisture content of the grain sampled at various depths showed the expected form, i.e. for a period no significant changes were seen because the drying front, moving up through the bin from the air inlet towards the surface, had not reached the zone from which moisture samples were taken. Then the moisture content at the lowest sampling position fell towards an equilibrium moisture value as the drying front passed. This process was repeated at each sampling position in turn as the drying front progressed toward the surface of the bin.

Temperature measurements in all four bins showed a similar pattern, in which the temperature of the ‘ambient’ air was increased due to the heating imparted by the fan, and then reduced by evaporative cooling as the air passed up through the damp grain. The temperature in the plenum ducts was between 3.6 and 4.0^{o}C hotter than the ambient temperature measured near the bins. This was a greater temperature rise than would normally be found in near ambient drying equipment and was due to the heating of the air by turbulence caused by right-angled bends in the inlet ducts. This meant that, in effect, drying was carried out with a heater constantly on.

The inlet air r.h., initially at about 75%, fell over the experiment to about 65%, while the exhaust remained above 95% throughout. The outlet r.h. varied with moisture content, the exhaust r.h. being 91% for the two driest bins and 97 and 98% for the

two wettest. The exhaust r.h. remained at this level until the drying front passed through the bed surface at which time it dropped to the level of the inlet r.h.

The average airflow through the bins was $32m^3$/min.

Table 1 gives counts of *P. verrucosum*, field and storage fungal species. Counts of field fungi, mainly *Cladosporium* and *Aureobasidium* spp, varied between 10^3 and 10^5 colonies per g at the start of the test and changed little. Counts of storage fungi, mainly *Penicillium* spp but also consistently including *Wallemia*, *Eurotium* and *Asperguillus* spp were of the order 10^2 colony forming units (c.f.u.) per g in the two bins at the lowest moisture content and rose to around 10^4 per g by the time drying was complete and in the two damper bins from 10^3/g to over 10^5/g by the time drying was complete. *P. verrucosum* was only detected in one sample at the start of the test.

Table 1. Counts of *Penicillium verrucosum*, field and storage fungal species in the four bins.

Initial moisture content (%)	Average count (log_{10} c.f.u. g^{-1})					
	P. verrucosum		Storage species		Field species	
	Initial	Final	Initial	Final	Initial	Final
19.9	0	1.7	2.9	4.0	3.6	4.5
21.4	0	2.4	3.8	5.4	4.9	4.5
23.3	1.8	3.9	3.0	4.0	4.7	4.4
23.9	0	4.3	3.6	5.9	5.1	4.7

Table 2 gives the final OTA levels and associated counts of *P. verrucosum*. The grain was sampled using two methods. Firstly, the grain was sampled using the regulatory sampling method (Commission Directive No. 2002/26/EC), which involves taking 100 samples of 100g of grain. Ten samples of 100g of grain were taken from each of ten columns. The samples from each column were analysed separately. Secondly, the grain was sampled at the surface and at a depth of 0.5m at five positions.

Table 2. Final OTA levels (ppm) and associated counts of *Penicillium verrucosum* (log_{10} c.f.u g^{-1}).

Moisture content of the grain			19.9	21.4	23.3	23.9
Regulatory Sampling from 10 columns		Ave (ppm)	0	0	0.08	3.83
		Nos +ve	0	0	4	8
		Range (ppm)	-	-	0.11-0.33	0.08-16.31
Samples taken from 5 locations	surface	Ave (ppm)	0.15	0	1.31	3.42
		Nos +ve	1	0	2	3
		Range (ppm)	0-0.75	-	0-3.35	0-16.1
		P.verrucosum	1.5	2.7	4.1	4.3
	0.5m	Ave (ppm)	0.37	0	1.14	1.78
		Nos +ve	1	0	1	3
		Range (ppm)	0-1.83	-	0-5.69	0-5.56
		P.verrucosum	1.9	0	3.8	4.3

The average level of *P. verrucosum* was 51c.f.u g^{-1} in the bin with a moisture content of 19.9%. This level of *P. verrucosum* was apparently associated with occurrence of OTA in only 1 in 5 samples. Where OTA did occur it was at less than 1ppb. Higher counts of *P. verrucosum* at the surface of the bin at 21.4% moisture content did not coincide with detection of OTA. At 0.5m, neither *P. verrucosum* nor OTA were detected.

P. verrucosum numbers reached around 10^4c.f.u g^{-1} in the damper bins by the time drying was complete, coinciding with 1 to 3 samples out of five containing OTA at an average of over 1ppb and with individual samples exceeding 5ppb.

The regulatory sampling method, taking 100 samples of 100g from 10 columns detected no OTA in the two driest bins whereas the individual samples from 5 positions taken at the surface and 0.5m did detect OTA. In the two bins at the higher level of moisture content, more columns produced positive OTA detections than did samples taken from 0 and 0.5m, but, as before, averages were higher using the smaller, non-regulatory samples. This suggests that taking 100 samples, as required for regulatory sampling, may not be necessary and that taking a smaller number of

samples from and near the surface may be all that is required to provide a satisfactory test for the presence of OTA.

The simulation, 'Storedry', calculated well the general drying behaviour and final moisture content, but underestimated the experimental drying time by about 20%. The possible reasons include that the exhaust air in the simulation was less saturated than in the experiment and the simulated drying zone was narrower, and airflow, which is very difficult to measure but a very important determinant of drying rate. The physical parameters were better predicted when the airflow was increased by 25%, so this expedient was used to allow the biodeterioration model to be correctly tested.

The prediction of biodeterioration, using a spoilage index based on the new model of safe time before OTA production may start, was found to be too rapid. Experimental data from Year 1 pointed to a safe storage time at least 2 times longer than predicted by the model, and possibly up to 6 times. One of the possible reasons for this large difference was that the Jonsson model was based on small scale experiments in which the wheat had been inoculated with *P. verrucosum*, the main cause of OTA in UK cereals. Although spores of this fungus were present in the grain sampled after drying in Year 1 experiment, very few spores were present in the grain sampled before drying started. This might be expected on freshly harvested grain as *P. verrucosum* is considered a storage fungus rather than a field fungus. However, it was considered possible that the low spore count of the grain before drying, possibly unusually low in the UK context, may have been responsible for the lower than predicted OTA levels after drying. Consequently, the experiment in Year 2 was designed around the use of an inoculation treatment of the damp wheat with spores of *P. verrucosum*.

Year 2

The same four bins as before were used with wheat at moisture contents of 17.2, 18.9 20.0 and 21.9%. The top 1m of each bin was segregated into nine $1m^3$ cells using a grid of plywood sheeting arranged vertically so as not to affect the airflow.

As each cell was loaded it was inoculated using suspensions of *P. verrucosum* spores, dripped into the grain using a watering can as it entered the cell. Three cells in each bin were inoculated at 1000 spores/g, three cells were inoculated at 10 spores/g and the final three cells were inoculated using water.

The grain was dried as in Year 1 and the temperature, airflow, relative humidity and static pressure were monitored as in Year 1. The grain was sampled twice weekly for moisture, mould and OTA analysis to follow the production of OTA.

Measurements of temperature, airflow, moisture content, relative humidity and static pressure followed a similar pattern to Year 1. The simulation model Storedry again gave good predictions of drying behaviour, though the same areas of difference as Year 1 were again apparent. Although time for the wettest layers to dry was well predicted using the experimental airflow, a better estimate of time above the moisture content suitable for fungal growth was obtained by increasing the measured airflow by 25%, as in Year 1.

Table 3 gives details of sample timing of samples analysed for fungal counts and OTA in relation to drying time. Samples were taken from each of the nine cells in each bin from the surface and from a depth of 0.5m.

Table 3. Timing of sampling.

Initial moisture content (%)	21.9	20.0	18.5	17.2
Experimental drying time (days)	29	24	26	20
Time when samples taken for OTA (days)	22,29	21,24	26	20
Time when samples taken for fungal counts (days)	11,22	11,21	11,22	13,16

Tables 4 and 5 show the counts of *P. verrucosum* from the surface and 0.5 m.

Table 4. Counts of *P. verrucosum* at the surface.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* (log_{10} c.f.u g^{-1})			
		21.9 %	20.0 %	18.5 %	17.2 %
1000	1	4.11	2.72	1.30	1.70
	2	4.31	2.48	3.00	0
10	1	4.11	3.11	2.43	1.78
	2	4.59	2.00	2.74	0
0	1	4.11	2.36	1.30	2.11
	2	3.85	2.30	1.78	0

Table 5. Counts of *P. verrucosum* at 0.5 m.

Level of inoculum (spores/g)	Sampling	Average count of *P. verrucosum* (log_{10} c.f.u g^{-1})			
		21.9 %	20.0 %	18.5 %	17.2 %
1000	1	5.01	2.91	2.30	2.32
	2	5.35	3.41	2.08	2.70
10	1	4.64	3.00	2.48	2.04
	2	4.67	2.30	2.60	0
0	1	4.63	2.46	2.11	1.30
	2	3.76	1.15	0	0

Counts for the three driest bins were small even in the bin with initial moisture content of 20%.

Where the average initial moisture content was 21.9% the counts were larger. In this case there is some evidence that the inoculation had an effect with the highest counts found at 0.5m in cells with the highest level of inoculum.

No OTA was detected in the incoming grain or in the two bins with the lowest moisture content when drying in those bins was complete. In the bin with an initial moisture

content of 20.0%, no samples from day 21 showed detectable OTA but one sample from day 24 was found to contain OTA at 0.4ppb, marginally above the limit of detection of 0.2ppb and well below the permitted level of 5ppb.

In the bin with highest initial moisture content, OTA was detected in 22 out of 36 samples. Although no samples exceeded the EC regulatory limit, this result shows that the safe life of the wheat has certainly been exceeded. Table 6 shows the average and maximum levels of OTA found at the surface and a depth of 0.5m in this bin.

Table 6. OTA in the bin with an initial moisture content of 21.9 %.

Position	Level of inoculum (spores/g)	Average level of Ochratoxin A (ppb)	Maximum level of Ochratoxin A (ppb)
Surface	1000	1.01	2.96
	10	0.60	3.10
	0	0.92	1.50
0.5 m	1000	0.56	2.40
	10	0.66	3.13
	0	0.17	0.50

The average level of OTA detected after 22 days was 0.6ppb and the average level after 29 days was 0.7ppb. The drying front had passed through the surface before either of the two sets of samples had been taken and so all the grain would have been below the moisture at which activity of the *P. verrucosum* fungus would occur and therefore the levels of OTA would not be expected to increase. However, there was no correlation between the OTA results for a given location between samples taken after 22 days and those taken after 29 days. By this stage the levels of OTA would not be expected to increase but neither would the levels be expected to decrease with time as OTA is stable. The lack of correlation suggests that the OTA was not uniformly distributed in the grain in each cell, otherwise point sampling would have given a consistent result.

No correlation between the level of inoculum and the level of OTA was found, nor was there a correlation between the count measurements for *P. verrucosum* and the level of OTA. That results from the three inoculation levels were not significantly different is

a positive result. If there had been a difference, it would have been necessary to know the amount of inoculum present in wheat in store in order to select the best drying strategy. Clearly this is not practicable.

Six months after drying three of the bins were sampled using the statutory method for OTA analysis. The level detected in the bin with the initial moisture content of 21.9% was 1.9ppb. No OTA was detected in the other bins.

Experimental data from Year 2 confirmed that the safe storage time predicted by the model was too conservative. For continuous ventilation drying, the data indicated a safe storage time between 2.1 and 4.3 times longer than predicted by the model.

Earlier simulations were re-run using a less conservative criterion for successful drying and results were compared with simulations using the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability. Of the 3980 simulations, 3337 dried within the time limit of which 47% were spoiled with the Fraser & Muir model and a spoilage index ≥1 while 42% were spoiled with the Jonsson model and a spoilage index ≥2. Based on this, the new model with the higher spoilage index was no more demanding than the Fraser & Muir model.

Scientific conclusions

1. The experiment used moisture contents and airflows that allowed OTA development in the wettest treatment, but prevented it in the lower moisture content treatments. It produced a good set of data, appropriate for use in validating both the physical and biodeterioration elements of the simulation model.
2. Neither the presence or the amount of OTA, or the counts of fungal colonies of *P. verrucosum* showed any significant relationship to level of inoculum.
3. The simulation, 'Storedry', gave good predictions of the general drying behaviour, final moisture content and time for the wettest layers, near the surface of the bed, to dry. For example, at 1m, the measured and predicted final moisture content of the four bins dried in year 1 agreed to within 0.5% moisture content.
4. The exhaust air in the simulation was less saturated than in the experiment. The relationship between wheat equilibrium moisture content and air relative humidity is responsible for this aspect of the simulation, so its accuracy at high relative humidity needs to be reviewed and improved.
5. The difference between the simulated and measured r.h. of the exhaust air led to the drying time being underestimated by 20%. A better estimate was obtained by

increasing the measured airflow by 25%. This allowed the biodeterioration model to be correctly tested.

6. The new model of safe time before OTA production may start was too conservative. For continuous ventilation drying, the data indicated a safe storage time between 2.1 and 4.3 times longer than predicted by the model. It should be noted that the new model has not been tested in intermittent ventilation, as used by most farm drying systems.
7. The criterion for successful drying, that spoilage index should not exceed 2, was applied to earlier simulation runs and results were compared with simulations using the reasonably well established Fraser & Muir model based on visible mould and significant loss of viability. The new model with the higher spoilage index was found to be no more demanding than the Fraser & Muir model.

Industrial conclusions

1. When drying by continuous ventilation, the risk of OTA appears no greater than the risk of visible mould or significant loss of viability.
2. For drying by continuous ventilation, the time for the drying front to pass through the bed does not need to be reduced. Hence, recommendations for bed depth and airflow rate in the Grain Storage Guide remain appropriate for continuous ventilation.
3. Drying rate does not need to depend on the amount of inoculum present.